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Heterogeneity of α_{2u}-globulin gene products in different translational systems, plasma and urine

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α_{2u}-Globulin, an androgen dependent rat urinary protein, displays considerable microheterogeneity. To explore whether this microheterogeneity of α_{2u}-globulin in male rat urine is related to the heterogeneity at the level of the genes encoding this protein, or whether it is due to post-translational processing we studied the α_{2u}-globulin mRNA translation products in rabbit reticulocyte and *Xenopus* oocytes. Comparison of the α_{2u}-globulin species produced in these two heterologous systems with those observed in plasma and urine indicates that the heterogeneity of this protein in urine is mainly due to heterogeneity at the level of the corresponding mRNAs.

Rat liver *Cell-free translation* *Urinary protein* *Isoelectric focusing* *Recombinant DNA* *Multigene family*

1. INTRODUCTION

α_{2u}-Globulin, an androgen-dependent rat urinary protein, is synthesized in the liver, secreted into the blood and excreted in the urine. Isoelectric focusing followed by immunofixation reveals considerable microheterogeneity of α_{2u}-globulin in male rat urine [1]. α_{2u}-globulin is encoded by a multigene family [2,3]; several of these genes are transcribed into mRNA [3,4]. To explore whether the microheterogeneity of α_{2u}-globulin in rat urine is related to the heterogeneity at the level of the genes encoding this protein, or whether it is due to post-translational processing we studied the α_{2u}-globulin mRNA translation products in rabbit reticulocyte lysate and *Xenopus* oocytes. Comparison of the α_{2u}-globulin species produced in these two heterologous systems with those observed in plasma and urine indicates that the heterogeneity of this protein in urine is mainly due to heterogeneity at the level of the corresponding mRNAs.

2. MATERIALS AND METHODS

2.1. *Isolation of poly(A)+ RNA and enrichment for α_{2u}-globulin mRNA sequences*

Poly(A)+ RNA was prepared from livers of adult male Wistar rats as in [5]. Enrichment for α_{2u}-globulin mRNA sequences was performed by hybridisation with an α_{2u}-globulin cDNA probe prepared as follows. Total poly(A)+RNA from livers of male rats was reverse-transcribed using avian myelobastosis virus reverse transcriptase (obtained from J.W. Beard). Second strand synthesis was carried out, with DNA polymerase I (Klenow fragment). After digestion with *Eco*RI and *Hin*dIII, fragments were fractionated by electrophoresis in agarose gel (low melting temperature). A size fraction containing fragments of 500–600 basepairs was excised and eluted, and subsequently ligated into pBR327 cleaved with *Eco*RI and *Hin*dIII. After transformation in *Escherichia coli* HB101, the male-specific clones were identified by colony hybridisation as in [6]. A

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530 basepair *Eco*RI *Hin*dIII insert of one of these male-specific clones was sequenced as in [7]. The nucleotide sequence was found to be identical to that of the corresponding fragment in the α_{2u}-globulin cDNA sequenced in [6].

To prepare single-stranded α_{2u}-globulin cDNA the 530 basepair fragment was transferred to M13mp9; 20 μg single-stranded DNA of the recombinant named M13u01 was bound to small (1 cm^2) nitrocellulose filters and hybridized with 250 μg total liver poly(A)$^+$ RNA in 50% formamide for 3 h at 42°C [8]. The filters were washed and α_{2u}-globulin mRNA was eluted by boiling for 1 min. The α_{2u}-globulin mRNA was recovered by precipitation with 2 vol. of ethanol.

Using this enriched α_{2u}-globulin mRNA, more than 50% of the radioactivity incorporated during heterologous translation in the reticulocyte lysate system (see further) could be precipitated with an anti-α_{2u}-globulin antiserum. Using an unprocessed poly(A)$^+$ preparation, the corresponding value averaged 4%.

2.2. *Heterologous translation*

Total or enriched mRNA was translated in rabbit reticulocyte lysate with added dog pancreas membranes (New England Nuclear translation and processing kit). Injection of *Xenopus* oocytes was performed as in [5]. Translation products were studied both in oocyte homogenate and in the medium after 48 h incubation.

2.3. *Analysis of translation products*

α_{2u}-Globulin was isolated from the translation mixtures using affinity chromatography as in [1]. The bed volume of the columns was reduced to 0.8 ml. α_{2u}-Globulin was eluted with 4 M $MgCl_2$, dialysed 24 h against saline and 24 h against 0.1 M ammonium sulfate, lyophylized and dissolved in phosphate-buffered saline. The microheterogeneity was studied by isoelectric focusing [1] followed by autoradiography. Male rat urine was applied on the same gels. The corresponding part of the gels was subjected to immunoprinting to identify the various forms of α_{2u}-globulin [1] and to permit comparison with the autoradiograms.

3. RESULTS AND DISCUSSION

α_{2u}-Globulin excreted in male rat urine displays

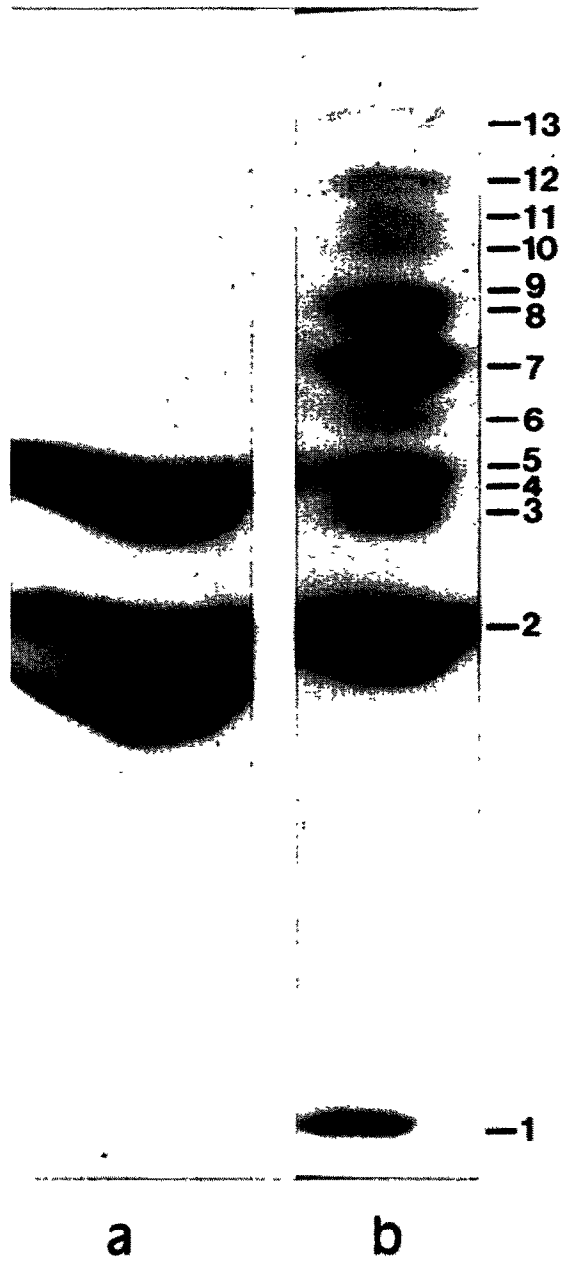

Fig.1. Microheterogeneity of α_{2u}-globulin in male rat urine and plasma. Male rat urine (diluted 1/2) and α_{2u}-globulin from male rat plasma, isolated by affinity chromatography, were subjected to isoelectric focusing on Ampholine polyacrylamide gel plates over pH 4–6.5. Immunofixation was performed using a rabbit antiserum directed against the purified major component (band 2) of α_{2u}-globulin in male rat urine as in [1]. Lane: a, α_{2u}-globulin isolated from male rat plasma; b, male rat urine. The holes are due to antigen excess.

considerable microheterogeneity [1]. To study whether this heterogeneity exists already in plasma, α_{2u}-globulin was purified from 500 ml male rat plasma by affinity chromatography [1]. Isoelectric focusing followed by immunofixation reveals that several α_{2u}-globulin species observed in male rat urine can also be demonstrated in plasma (fig.1). Some quantitative differences may be observed, however. It is conceivable that these are related to differences in the clearance rates of various α_{2u}-globulin species. To further explore whether this heterogeneity of α_{2u}-globulin in urine and plasma is a consequence of the heterogeneity at the level of the genes coding for this protein, we analyzed and compared the translation products of total liver poly(A^+) RNA and enriched α_{2u}-globulin mRNA in rabbit reticulocyte lysate and *Xenopus* oocytes. Fig.2 shows that the α_{2u}-globulin synthesized in these heterologous systems also displays remarkable microheterogeneity. No differences are observed between α_{2u}-globulin species produced using total poly$(A)^+$ RNA or α_{2u}-globulin mRNA. Moreover, the heterogeneity is essentially identical in rabbit reticulocyte lysate, *Xenopus* oocyte homogenate and *Xenopus* oocyte medium suggesting that it is related to heterogeneity of the added α_{2u}-globulin mRNA and not to posttranslational modifications. One species (band 1) with an isoelectric point of about 6.2, however, is easily seen in rat urine and *Xenopus* oocyte medium but barely visible in the other systems. This particular product might be the result of some form of posttranslational processing. Finally, at least 7 species of α_{2u}-globulin observed in urine are also produced

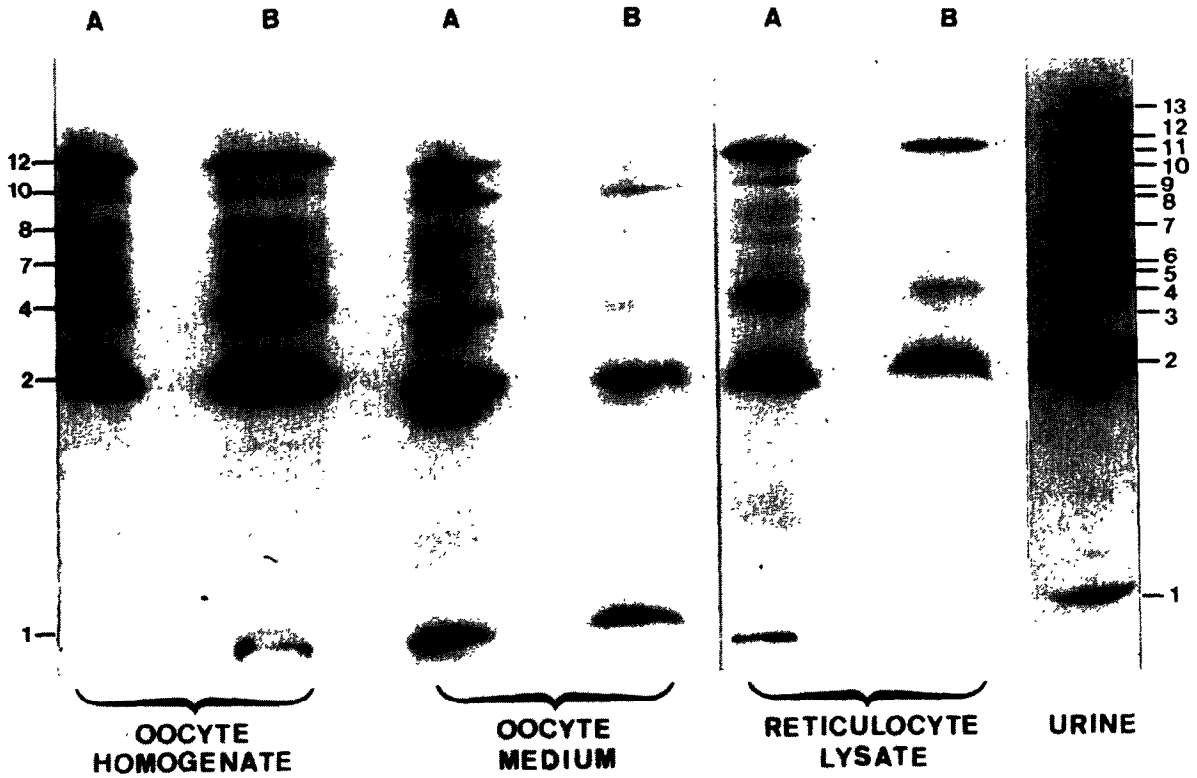

Fig.2. Microheterogeneity of α_{2u}-globulin synthesized in rabbit reticulocyte lysate and *Xenopus* oocytes. Male rat liver poly $(A)^+$ RNA (lanes A) or α_{2u}-globulin mRNA, enriched as described in section 2 (lanes B) were translated in rabbit reticulocyte lysate and *Xenopus* oocytes. α_{2u}-Globulin was isolated from the lysate, the oocyte homogenate and the oocyte medium by affinity chromatography and subjected to isoelectric focusing as described in fig.1. α_{2u}-Globulin was visualized by autoradiography. Male rat urine (diluted 1/2) was applied to the same gel and the α_{2u}-globulin species in this part of the gel were localized by immunofixation using an α_{2u}-globulin-directed antiserum.

in the heterologous translation systems. It is concluded from these data that the heterogeneity of α_{2u}-globulin in male rat urine already exists at the level of the α_{2u}-globulin mRNA and presumably reflects the heterogeneity at the level of the corresponding genes [3].

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